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Assessment of the Potential Use of Recombinant Baculovirus-expressed Lassa Virus Nucleoprotein as a serodiagnostic antigen

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Abstract

Lassa virus is an arenavirus causing a disseminated systemic primary viral infection. This virus causes Lassa fever which is a viral hemorrhagic fever endemic in West Africa and is responsible for the deaths of thousands of people each year. There is a possibility for the Lassa virus to be introduced into the US and used as a biological weapon with the potential to harm a large-scale population. Because of increasing international travel, a sizeable burden from the disease, and its potential use for biological warfare, it is necessary to develop sensitive diagnostic assays to accurately detect virus infections and mitigate against potential introduction and spread. The objective of this project was to produce recombinant Lassa virus nucleoprotein (N), the most abundant and immunogenic structural protein of the virus, using a recombinant baculovirus expression system and assess its use as an antigen to detect Lassa antibodies in infected hosts. Using the eukaryotic expression system, the gene encoding the N protein was cloned in donor plasmid, which was used to create a recombinant bacmid. The latter was used to transfect Spodoptera frugiperda (SF9) cells to rescue the recombinant baculovirus and express the target protein. The recombinant proteins were purified by affinity chromatography using nickel columns, and specific reactivity was assessed by western blot using anti-Lassa virus hyperimmune mouse ascitic fluid, mouse anti-Lassa monoclonal (ABE419) and mouse anti-Lass monoclonal (ABE420) antibodies. An estimated 63kDa recombinant N protein was overexpressed and authenticated via specific reactivity with the anti-Lassa virus hyperimmune mouse ascetic fluid but not with the monoclonal antibodies. Manifestation of specific immunoreactivity with the Lassa antiserum suggests the ability of the N protein to be recognized by host-specific Lassa antibodies and thus potentially serve as a serodiagnostic antigen for detection and seroepidemio-surveillance of Lassa fever in endemic and non-endemic regions. Future studies would entail evaluating the use of the recombinant antigen in ELISA to investigate the seroepidemiology of Lassa fever among febrile patients in endemic countries in West Africa.

Introduction

Lassa fever is a viral hemorrhagic fever endemic in regions of West Africa (Figure 1) and has the potential to kill tens of thousands of people each year. It is caused by the Lassa virus which is the most genetically diverse of the arenaviridae family. Lassa viruses are enveloped, single-stranded bi-segmented, ambi-sense RNA viruses (Figure 2). The large segment encodes a small zinc-binding protein (Z) and the RNA polymerase (L). The small segment encodes the N and the surface glycoprotein precursor (GP), which is proteolytically cleaved into the envelope glycoproteins GP1 and GP2.

Figure 1. Distribution of Lassa fever in West Africa

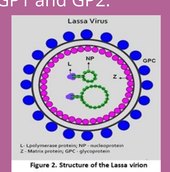

Figure 2. Structure of the Lassa virion

The natural reservoir host for Lassa virus is the African soft-furred rat which is found throughout the West African region. It is transmitted to humans through direct contact, inhalation or ingestion of infected rat excreta, or person to person via contact with infected body secretions. The symptoms of Lassa fever can be nonspecific mimicking those of other endemic infections which makes a clinical diagnosis difficult. There is a possibility that Lassa virus could be used as a biological weapon especially during civil unrest. Considering the fact that the efficacy decreases following onset of the disease, the World Health Organization in 2014 called for development of early diagnostic tests for Lassa fever. In this study, the viral nucleoprotein, the most immunogenic protein of Lassa virus was recombinantly expressed using a baculovirus expression system. The authenticity of the recombinant protein was subsequently evaluated for reactivity against a set of mouse anti-Lassa virus monoclonal antibodies as well as mouse anti-Lassa virus hyperimmune ascitic fluid. Herein, we report that the recombinant nucleoprotein exhibited specific immunoreactivity with the anti-Lassa virus hyperimmune ascitic fluid indicating its potential use for development of serodiagnostic tests.

Methodology

Cloning and Construction of Recombinant Bacmid

- The Lassa Fever N gene was amplified from recombinant pUC57-LassaN plasmid by high fidelity PCR
- The PCR product was purified (Figure 3) and cloned into pFastBac plasmid creating a donor plasmid, pFastBacN,
- The plasmids were purified using the QIAprep Spin Miniprep Kit.
- The accuracy of the N sequence in the recombinant pFastBacN was confirmed by restriction enzyme analysis using XhoI and by DNA sequencing.
- The recombinant pFastBacN was used to create recombinant bacmid (Figure 4)

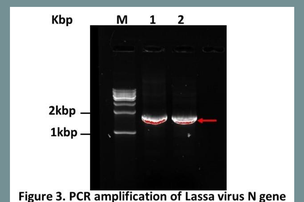

Figure 3. PCR amplification of Lassa virus N gene

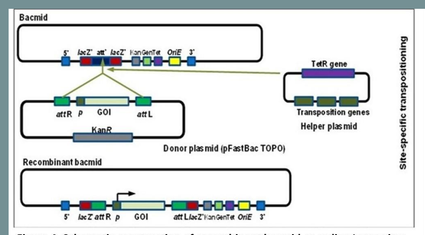

Figure 4. Schematic construction of recombinant bacmid encoding Lassa virus N gene

Lassa N Protein Expression and Purification

- Recombinant bacmid was used to transfect *Spodoptera frugiperda* (Sf9) cells to rescue a recombinant baculovirus.
- Recombinant baculovirus passage 2 (P2) was used to express recombinant Lassa N protein in Sf9 cells
- Recombinant proteins were purified by affinity Ni-NTA column chromatography.
- The purified protein was dialyzed overnight against PBS and concentration was measured using Nanodrop

Western Blot Analysis

- The purified protein was run through a polyacrylamide gel in 1X MOPS running buffer,
- Protein was transferred onto PVDF membrane according to standard protocol.
- The membranes were probed with anti-HIS (C-terminal)-HRP monoclonal antibody, and
- With monoclonal mouse Anti-Lassa antibodies and Anti-Lassa virus hyperimmune mouse ascitic fluid (1:2000; 1:4000; 1:8000; 1:16000).
- The membranes were probed with goat anti-mouse IgG-HRP (1:5000).
- Detection was performed using ECL chemiluminescent detection reagent and visualized using a bioRad imager.

Results

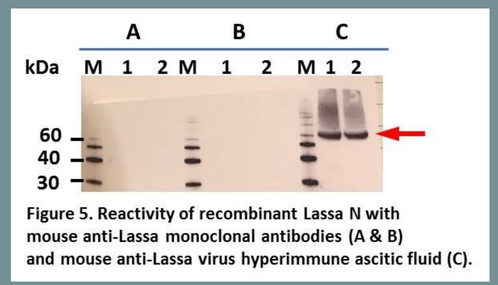

Figure 5. Reactivity of recombinant Lassa N with mouse anti-Lassa monoclonal antibodies (A & B) and mouse anti-Lassa virus hyperimmune ascitic fluid (C).

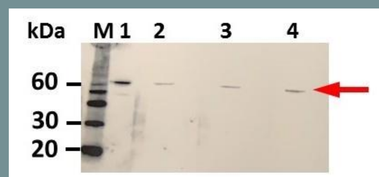

Figure 6. Reactivity of recombinant Lassa N with various dilutions of mouse anti-Lassa virus hyperimmune ascitic fluid. 1=1:2,000; 2=1:4,000; 3=1:8,000; 4=1:16,000

- Expression of recombinant Lassa virus N protein of the expected molecular size was detected using anti-His (C-terminal) monoclonal antibody
- Authenticity of the recombinant Lassa virus N protein was confirmed via specific reactivity with anti-Lassa virus the hyperimmune ascitic fluid (Figure 5)
- The hyperimmune ascitic fluid detected an estimated 62 kDa protein which corresponded with the expected molecular size
- The recombinant protein exhibited specific reactivity anti-Lassa antibodies in high dilutions (up to 16,000-fold) of the hyperimmune ascitic fluid (Figure 6)
- High antibody concentration or activity appeared to correlate with signal intensity (Figure 6; lane 1 versus lanes 2-4)

Discussions

- The Lassa N gene was successfully cloned and expressed.
- The recombinant N protein showed specific reactivity with ascitic fluid obtained from hyperimmune mice experimentally infected with Lassa virus.
- The detection of anti-Lassa virus specific antibodies at high dilutions suggests the possibility of developing a sensitive serodiagnostic test.
- This data suggests the baculovirus expressed Lassa N protein can serve as a useful diagnostic antigen for serological testing of Lassa fever virus infections.
- Recombinant baculovirus-expressed Lassa N protein contains antigenic epitopes that are reactive with antibodies raised against Lassa virus in an animal host.
- The findings represent a strong indication of the utility of the recombinant antigen for development of sensitive and specific serodiagnostic tests that could be used for both seroepidemiological and serosurveillance purposes.

References

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Acknowledgements

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